REMARKS

Claims 1-7 and 14-29 are presently pending. Claims 1, 3, 16, 21 and 26-29 are amended. Support for recitation of "wherein the solvent is tri-n-butyl phosphate" is found in the Specification as filed at page 5, lines 6-7. Support for recitation of "wherein a fraction of the thrombin has a specific activity of at least 2000 International Units per mg of protein" in Claims 1, 3, 16 and 21 is found in the Examples of the Specification as filed (e.g., Examples 1-3), which disclose production of a thrombin product having greater than at least 2000 International Units per mg of protein. No new matter has been added herewith. The following addresses the substance of the Office Action.

Obviousness

Claims 1-6, 14, 16-19, 21-24 and 26-29 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Kraus et al. (U.S. Patent No. 5,143,838) in view of Anderle et al. (U.S. Application Publication No. 2003/0133829). The basis of the rejection centers on the assertion that the skilled person would combine the solvent/detergent (S/D) inactivating step of Anderle et al. with the conversion of prothrombin to thrombin on an anion exchange column as disclosed by Kraus et al. The process disclosed in Anderle involves the use of a detergent in combination with a solvent, wherein the solvent is a carboxylic acid ester. Anderle et al. indicates that, with this specific combination of a carboxylic acid ester and detergent, pathogens in a protein solution are effectively inactivated while the protein activity is substantially fully preserved (see paragraph [0014] of Anderle). In contrast to Anderle et al., the S/D inactivation step in the presently amended claims uses tri-n-butyl phosphate as a solvent, and not a carboxylic acid ester, as was used in Anderle et al.

Moreover, Anderle et al. teaches away from the presently claimed method of inactivating virus with a conventional S/D treatment prior to activation of prothrombin to yield thrombin. In particular, Anderle teaches at paragraph [0005] that there is a serine protease group, which includes the prothrombin complex of coagulation factors, which are sensitive to, and at least partially deactivated by conventional S/D methods. For these proteins, Anderle recommends that detergent alone be used at high concentrations (i.e., without solvent such as tri-n-butyl phosphate). In contrast, the presently claimed methods are able to utilize a S/D treatment step prior to activation of prothrombin because the methods also include a column washing step,

wherein the solvent and detergent reagents used to inactivate virus are removed prior to subsequent activation of prothrombin to thrombin.

The Examiner cited paragraph [0058] of Anderle et al. at the final paragraph on page 3 of the Official Action to assert that Anderle et al. teaches that the order of steps is flexible with regard to "purification" and "enrichment" of proteins. However, the Examiner has incorrectly equated the term "enrichment," as used by Anderle et al., with the term "activation" as it pertains to activation of prothrombin. Anderle et al. uses the term "enrichment" in reference to purification, concentration or isolation of a protein (e.g., by chromatographic or precipitation methods). In contrast "activation" of a protein refers to a chemical process which changes the protein structure itself. In the present case, prothrombin is activated when Factor X proteolytic cleaves the precursor protein to yield active thrombin. Thus, the teachings of Anderle et al. specifically advise against the presently claimed method, wherein a solution containing Factor X, and optionally prothrombin, are treated with solvent and detergent prior to activation of the prothrombin to yield thrombin.

The Applicants previously argued that there is no disclosure in Kraus et al. that virus inactivation could be carried out on prothrombin prior to activation to yield thrombin, which is consistent with the knowledge of the person skilled in the art. In response, the Examiner stated that Kraus suggests that the timing of the S/D treatment is flexible and can be accomplished by treating the plasma or plasma fractions either before or after the thrombin is isolated. However, Kraus et al. provides no evidence or teaching that S/D treatment can be performed before the prothrombin is activated to thrombin. Notably, the relevant disclosure at column 3, lines 1-7 of Kraus et al. specifies that "before or after the thrombin is isolated from the plasma or plasma fractions" the batch can undergo S/D treatment. This is in contrast to the wording used at column 2, line 43 of Kraus et al., which specifies that the prothrombin (Factor II) is preferably "activated" into thrombin with Ca^{2+}. The skilled person would understand the term "activated" to refer to the chemical conversion of prothrombin into thrombin, whereas the term "isolated" would be understood by the skilled person as referring to the separation of one component from another. Thus, when Kraus et al. refers to virus inactivation before or after thrombin isolation, it is clear that this means before or after activated thrombin has been separated from other components, not before or after the thrombin is generated from prothrombin.

As mentioned in the previous response by the Applicants, the Specification specifically teaches at page 2, lines 24-29 that viral inactivation by S/D treatment should be carried out after thrombin has been activated, otherwise factors such as phospholipids, which are necessary for the activation reaction, would be eliminated; See, also, Michalski et al. (U.S. Patent No. 5,304,372; submitted herewith along with an IDS). In addition, as discussed above, the skilled artisan would have been discouraged from practicing the presently claimed method by Anderle et al., which teaches against using a conventional S/D treatment prior to activation of prothrombin since prothrombin is sensitive to, and at least partially deactivated by conventional S/D methods.

The Examiner cited Ralston et al. (US 6,245,548) as evidence that prothrombin can undergo a viral inactivation step prior to activation to thrombin. However, Ralston does not refer specifically to virus inactivation. Although both solvent and detergent are present in Example 1 of Ralston, the reference does not state that the material has undergone a virus inactivation treatment. The skilled person knows that effective S/D virus inactivation requires: (a) adequate concentrations of solvent and detergent; (b) adequate temperature; and (c) adequate incubation time. Ralston does not teach that such conditions were achieved or that virus inactivation was intended. In addition, Ralston states that the solvent and detergent present in the mixture interfere with thrombin activation from prothrombin and render it ineffective. Referring to column 4, lines 5-11 of Ralston, multiple polyethylene glycol (PEG) protein precipitations are required to remove inhibitors of prothrombin activation (i.e., detergent and sovent) before prothrombin can be activated if it has previously been in the presence of solvent and detergent. Moreover, before the activated thrombin could be used as a pharmaceutical product, it would be disadvantageously necessary to introduce another processing step (with consequential loss of yield) to remove the high concentration of PEG. Accordingly, Ralston is consistent with other references that teach that the presence of solvent and detergent inhibits activation of prothrombin to thrombin.

The Examiner cited Metzner as disclosing S/D treatment of prothrombin prior to activation to thrombin. However, column 2, lines 61-67 of Metzner merely refer to virus inactivation "by pasteurization or another known method." This cannot be considered as a teaching that thrombin may be activated after solvent/detergent virus inactivation of a prothrombin complex.

Similarly, the Examiner cited Karges, which indicates at column 2, lines 15-16 that, instead of pasteurization, viruses can be inactivated "in any other suitable way" prior to activation of the prothrombin complex to thrombin. However, S/D treatment is not mentioned. It is further noted that both examples disclosed in Karges involve the mixing of pasteurized prothrombin with 20 U/ml of human thrombin. Thus, Karges teaches the skilled person that the virus inactivated prothrombin complex is activated to thrombin in the presence of exogenous thrombin added to the reaction mixture.

Unexpected Results

The methods of the presently claimed invention provide unexpected results in view of Kraus et al. and Anderle et al. Based on the teachings of Anderle et al., one of skill in the art would predict that treating a prothrombin preparation with a conventional S/D treatment prior to activation of the prothrombin to yield thrombin would produce a thrombin preparation having a lower specific activity than that seen by Kraus et al., given that Anderle et al teaches that prothrombin is partially deactivated by such conventional S/D methods. On the contrary, the specific activity of the thrombin preparations generated by the presently claimed methods surpasses that of preparations generated using the method of Kraus et al. The Kraus reference discloses that a specific activity (purity) of approximately 800 to 1400 units per mg of protein can be directly obtained using the methods disclosed therein. See Kraus et al. at column 2, lines 61-67. In order to achieve a specific activity of more than 2000 units per mg of protein, Kraus et al. acknowledges that additional purification steps, such as cation exchange must be conducted. In contrast, the Examples disclosed in the present specification describe the production of a product having greater than at least 2000 units per mg of protein without the need for cation exchange chromatography or other purification steps, which can diminish yield. Claims 1, 3, 16 and 21 are amended to specifically recite that a fraction of the thrombin has a specific activity of at least 2000 International Units per mg of protein.

Moreover, the presently claimed invention provides a high concentration of thrombin, which is surprisingly better than that achieved by Kraus et al. Kraus et al. does not directly specify the concentration of the thrombin obtained in the process disclosed therein. However column 2, line 58 of Kraus et al. indicates that the concentration can be increased to more than 100 units per mL by known methods, e.g., ultrafiltration. Thus, the concentration that is directly

obtained from the methods described by Kraus et al. are necessarily lower than 100 units per mL. Such concentration methods are used in the pharmaceutical industry, but are cumbersome, expensive and time-consuming. Such a step would eliminate the benefits claimed in Kraus et al. of single step processing from plasma or plasma fraction. In contrast to the relatively low concentrations obtained by Kraus et al., as discussed in the Applicants previous response, the presently claimed invention provides a high thrombin concentration of more than 900 units per mL without the need for further processing steps.

In conclusion, the presently claimed methods provide a novel means to prepare thrombin from prothrombin, after pre-treatment of prothrombin with detergent to inactivate virus. The process yields both an unexpectedly high purity (Specific Activity > 2000 IU per mg of protein) and unexpectedly high concentration of thrombin. In contrast, extra steps are needed to produce thrombin by the method of Kraus et al. to achieve the same purity and potency as that obtained using the process of the presently claimed invention incur a yield penalty, which would eliminate any upstream yield benefit. The necessary extra steps undermine the notion that the process of Kraus et al. has overcome the problem of multiple step processes for the preparation of thrombin. Based on the foregoing remarks, the claimed methods of the present application provide unexpected advantages over Kraus et al. in view of Anderlie et al. Even had a proper *prima facie* showing of obviousness been set forth by the references cited, the unexpected results of high specific activity and high concentration of thrombin prepared by the presently claimed invention rebut any such showing.

Claims 7, 15, 20 and 25 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Kraus et al. (*supra*) in view of Anderle et al. (*supra*) in further view of Kingdom et al. (U.S. Patent No. 5,354,682) and Heimburger et al. (U.S. Patent No. 6,346,277). However, since Claims 7, 15, 20 and 25 are ultimately dependent on Claims 1, 3, 16 and 21, respectively, they are not obvious in light of the remarks above. The disclosures of Kingdom et al. and Heimburger et al. do not provide additional information beyond the disclosures of Kraus et al. and Anderle et al. that would make the claimed methods obvious. Accordingly, the Applicants respectfully request removal of the rejections under 35 U.S.C. § 103(a).

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

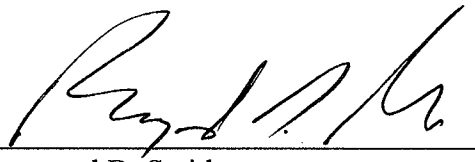
In view of Applicants' amendments to the Claims and the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: May 20, 2009

By: 

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